

Application No. 10/758,488  
Amdt. dated December 21, 2005  
Reply to Office action of November 21, 2005

**Listing of Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

- Claim 1. (Original) An isolated thioaptamer that mediates gene silencing.
- Claim 2. (Original) The thioaptamer of claim 1, further comprising a terminal 3' hydroxyl group.
- Claim 3. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises ribonucleotides.
- Claim 4. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises deoxyribonucleotides.
- Claim 5. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises one or more of the following: rATP( $\alpha$ S), rUTP( $\alpha$ S), rGTP( $\alpha$ S), rCTP( $\alpha$ S), rATP( $\alpha$ S<sub>2</sub>), rUTP( $\alpha$ S<sub>2</sub>), rGTP( $\alpha$ S<sub>2</sub>) or rCTP( $\alpha$ S<sub>2</sub>).
- Claim 6. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises from about 21 to about 25 nucleotides.
- Claim 7. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
- Claim 8. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises an imperfect complementarity match to a target gene and gene silencing occurs by repressed translation of mRNA to protein.
- Claim 9. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
- Claim 10. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises a portion of a RNA-induced silencing complex (RISC) complex.
- Claim 11. (Original) The thioaptamer of claim 1, wherein the thioaptamer is produced by a DICER complex.
- Claim 12. (Currently amended) The thioaptamer of claim 1, wherein the thioaptamer comprises a short interfering RNA (siRNA); ~~a micro-interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA).~~
- Claim 13. cancelled
- Claim 14. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises a double stranded thioaptamer of about 21 to about 25 nucleotides long.
- Claim 15. (Original) The thioaptamer of claim 1, wherein the thioaptamer comprises a single-stranded thioaptamer that is about 15 to about 22 nucleotides long.

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Claim 16. (Original) The thioaptamer of claim 1, wherein gene silencing is defined further as degradation of an mRNA transcript that is cleaved in the presence of the thioaptamer before it can express a protein.

Claim 17. (Original) The thioaptamer of claim 1, wherein gene silencing is defined further as regulation of translation when the thioaptamer binds an mRNA transcript at or about its 3'UTR.

Claim 18. (Withdrawn) A method of producing a mature thioaptamer of from about 21 to about 23 nucleotides in length comprising the steps of:

combining a double-stranded precursor thioaptamer with a soluble extract that mediates gene silencing, thereby producing a precursor-extract mixture; and

maintaining the precursor-extract mixture under conditions in which the double-stranded thioaptamer is processed to the mature thioaptamer of from about 21 to about 23 nucleotides in length.

Claim 19. (Withdrawn) The method of claim 18, further comprising isolating the thioaptamer of from about 21 to about 23 nucleotides from the precursor-extract mixture.

Claim 20. (Withdrawn) The method of claim 18, further comprising the step of determining the sequence of the mature thioaptamer and the location of one or more thio-modifications to the mature thioaptamer.

Claim 21. (Withdrawn) The method of claim 18, further comprising the steps of:

determining the sequence of the mature thioaptamer and the location of one or more thio-modifications to the mature thioaptamer; and

chemically synthesizing the mature thioaptamer.

Claim 22. (Withdrawn) A mature thioaptamer of about 21 to about 23 nucleotides produced by the method of claim 18.

Claim 23. (Withdrawn) A method of mediating gene silencing of a target gene in a cell or organism comprising the steps of:

introducing a thioaptamer of from about 21 to about 23 nucleotides in length into the cell or organism; and

maintaining the cell or organism under conditions in which gene silencing occurs, thereby mediating expression of the target gene in the cell or organism.

Claim 24. (Withdrawn) The method of claim 23, wherein thioaptamer is optimized for RNase H degradation of the message.

Claim 25. (Withdrawn) The method of claim 23, wherein the target gene encodes a viral gene.

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- Claim 26. (Withdrawn) The method of claim 23, wherein the target gene encodes a cellular gene.
- Claim 27. (Withdrawn) The method of claim 23, wherein gene silencing is defined further as degradation of an mRNA transcript of the target gene that is cleaved in the presence of the thioaptamer before it can express a protein;
- Claim 28. (Withdrawn) The method of claim 23, wherein gene silencing is defined further as regulation of translation of the target gene when the thioaptamer binds an mRNA transcript of the target gene at or about its 3'UTR.
- Claim 29. (Withdrawn) The method of claim 23, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to the target gene and gene silencing occurs by mRNA cleavage.
- Claim 30. (Withdrawn) The method of claim 23, wherein the thioaptamer comprises an imperfect complementarity match to the target gene and gene silencing occurs by repressed translation of mRNA to protein.
- Claim 31. (Withdrawn) The method of claim 23, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to the target gene and gene silencing occurs by mRNA cleavage.
- Claim 32. (Withdrawn) A knockdown cell or organism generated by the method of claim 23.
- Claim 33. (Withdrawn) The knockdown cell or organism of claim 32, wherein the cell or organism mimics a disease.
- Claim 34. (Withdrawn) The knockdown cell or organism of claim 32, wherein the cell comprises a stem cell.
- Claim 35. (Withdrawn) A method of examining the function of a gene in a cell or organism comprising the steps of:
- introducing a thioaptamer of from about 21 to about 23 nucleotides that targets an mRNA of the gene for gene silencing into the cell or organism, thereby producing a test cell or test organism;
- maintaining the test cell or test organism under conditions under which gene silencing of mRNA of the gene occurs, thereby producing a test cell or test organism in which mRNA of the gene is silenced; and
- observing the phenotype of the test cell or test organism against an appropriate control cell or control organism to provide information about the function of the gene.

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Claim 36. (Withdrawn) A method of assessing whether a gene product is a suitable target for drug discovery comprising the steps of:

introducing an RNA thioaptamer that mediates gene silencing of from about 21 to about 25 nucleotides into a cell or organism under conditions in which gene silencing of an mRNA for the target gene results in decreased expression of the gene; and

determining the effect of the decreased expression of the gene on the cell or organism, wherein if decreased expression has an effect, then the gene product is a target for drug discovery.

Claim 37. (Original) A pharmaceutical composition comprising a thioaptamer of from about 21 to about 25 nucleotides that mediates thioaptamer gene silencing and an appropriate carrier.

Claim 38. (Withdrawn) A method of identifying target sites within an mRNA that are efficiently targeted for gene silencing, comprising the step of:

combining an RNA thioaptamer corresponding to a sequence of a labeled mRNA to be degraded under conditions in which labeled mRNA is degraded.

Claim 39. (Withdrawn) The method of claim 38, further comprising the step of identifying one or more sites in the mRNA that are efficiently cleaved.

Claim 40. (Withdrawn) The method of claim 38, wherein the RNA thioaptamer is defined further as a thioaptamer library.

Claim 41. (Withdrawn) The method of claim 38, wherein the RNA thioaptamer is defined further as a pool of thioaptamers from a thioaptamer library.

Claim 42. (Withdrawn) A method of identifying target sites within an mRNA that are efficiently targeted for gene silencing, comprising the step of:

combining an RNA thioaptamer corresponding to a sequence of a labeled mRNA under conditions in which labeled mRNA is not degraded and the protein level is reduced.

Claim 43. (Withdrawn) The method of claim 42, wherein the RNA thioaptamer is defined further as a thioaptamer library.

Claim 44. (Withdrawn) The method of claim 42, wherein the RNA thioaptamer is defined further as a pool of thioaptamers from a thioaptamer library.

Claim 45. (Withdrawn) A combinatorial thioaptamer library comprising:

two or more unique thioaptamers that comprise a combination of backbone modifications and sequence that

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mediates gene silencing of an mRNA to which it corresponds.

- Claim 46. (Withdrawn) The library of claim 45, wherein the thioaptamers are attached covalently to one or more beads.
- Claim 47. (Withdrawn) The library of claim 46, wherein the beads are polystyrene/polydivinyl benzene copolymer.
- Claim 48. (Withdrawn) The library of claim 45, wherein the thioaptamers comprise one or more phosphorothioate linkages.
- Claim 49. (Withdrawn) The library of claim 45, wherein the thioaptamers comprise one or more phosphorodithioate linkages.
- Claim 50. (Withdrawn) The library of claim 45, wherein the thioaptamers comprise one or more methylphosphonate linkages.
- Claim 51. (Withdrawn) The library of claim 45, wherein the thioaptamers comprises one or more of the following: rATP( $\alpha$ S), rUTP( $\alpha$ S), rGTP( $\alpha$ S), rCTP( $\alpha$ S), rATP( $\alpha$ S<sub>2</sub>), rUTP( $\alpha$ S<sub>2</sub>), rGTP( $\alpha$ S<sub>2</sub>) and rCTP( $\alpha$ S<sub>2</sub>).
- Claim 52. (Withdrawn) The library of claim 45, wherein the thioaptamer comprises a viral protein sequence.
- Claim 53. (Withdrawn) The library of claim 45, wherein the thioaptamer comprises a genomic sequence.
- Claim 54. (Withdrawn) The library of claim 45, wherein the thioaptamer comprises an expressed sequence.
- Claim 55. (Withdrawn) The library of claim 45, wherein each of the thioaptamers further comprise a colorimetric agent.
- Claim 56. (Withdrawn) The library of claim 45, further comprising the complementary strand to the thioaptamer.
- Claim 57. (Withdrawn) The library of claim 45, wherein the thioaptamers is created by a split and pool combinatorial synthesis chemistry.
- Claim 58. (Withdrawn) The library of claim 45, wherein the thioaptamer library comprises double stranded thioaptamers with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
- Claim 59. (Withdrawn) The library of claim 45, wherein the thioaptamer library comprises

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thioaptamers with imperfect complementarity matches to a target gene and gene silencing occurs by repressed translation of mRNA to protein.

Claim 60. (Withdrawn) The library of claim 45, wherein the thioaptamer comprises library single-stranded thioaptamers with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.

Claim 61. (Withdrawn) A one-bead, one-thioaptamer combinatorial library comprising:  
two or more beads, wherein attached to each bead is a unique thioaptamer comprising a single unique sequence, wherein each unique thioaptamer comprises a unique mix of modified and unmodified nucleotides and wherein the thioaptamer mediates gene silencing of an mRNA to which it corresponds.

Claim 62. (Withdrawn) A one-bead, one-thioaptamer combinatorial library comprising:  
two or more beads, wherein attached to each bead is a unique thioaptamer comprising an imperfect complementarity match to a target gene to form a thioaptamer-bead, wherein each unique thioaptamer-bead comprises a unique mix of modified and unmodified nucleotides and wherein the thioaptamer mediates gene silencing of an mRNA to which it has imperfect complementarity.

Claim 63. (Withdrawn) A combinatorial library comprising:  
a bead library of thioaptamer libraries, wherein each bead comprises a thioaptamer library of imperfect complementarity to a target sequence for gene silencing.

Claim 64. (Withdrawn) A method for reducing the expression of a gene in a cell, comprising the steps of:

selecting a thioaptamer that mediates gene silencing of the gene to which it corresponds; and  
introducing the thioaptamer into the cell, wherein the thioaptamer mediates RNA interference of a targeted sequence.

Claim 65. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises from about 21 to about 25 nucleotides.

Claim 66. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises a double stranded thioaptamer with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.

Claim 67. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises an imperfect complementarity match to a target gene and gene silencing occurs by repressed translation of mRNA to protein.

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- Claim 68. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises a single-stranded thioaptamer with perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage.
- Claim 69. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises a portion of a RNA-induced silencing complex (RISC) complex.
- Claim 70. (Withdrawn) The method of claim 63, wherein the thioaptamer is produced by a DICER complex.
- Claim 71. (Withdrawn) The method of claim 63, wherein the thioaptamer comprises a short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA).
- Claim 72. (Withdrawn) The method of claim 63, wherein the thioaptamer is further defined as a thioaptamer precursor that comprises a long dsRNA, an about 70 nucleotide stem-loop RNA (shRNA) or an about 70 nucleotide stem-loop RNA (shRNA).
- Claim 73. (Withdrawn) The method of claim 63, wherein the targeted sequence is selected from the group consisting of: markers, splice acceptors, splice donors, IRES, recombinase sites, promoters, ori sequences, cloning sites, and intervening sequence.
- Claim 74. (Withdrawn) The method of claim 63, wherein the targeted sequence comprises a viral sequence.
- Claim 75. (Withdrawn) The method of claim 63, wherein the targeted sequence comprises an autologous sequence.
- Claim 76. (Withdrawn) The method of claim 63, wherein the targeted sequence comprises a heterologous sequence.
- Claim 77. (Withdrawn) The method of claim 63, wherein the cell is a mammalian cell.
- Claim 78. (Withdrawn) The method of claim 63, wherein the cell is a human cell.
- Claim 79. (Withdrawn) The method of claim 63, wherein the cell is a stem cell.
- Claim 80. (Withdrawn) The method of claim 63, wherein the thioaptamer is an antisense molecule.
- Claim 81. (Withdrawn) The method of claim 63, wherein the thioaptamer is a ribozyme.
- Claim 82. (Withdrawn) The method of claim 63, wherein the thioaptamer is a double-stranded RNA (dsRNA).
- Claim 83. (Withdrawn) The method of claim 63, wherein the gene is associated with a disease or

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disorder.

Claim 84. (Withdrawn) A method for attenuating expression of a target gene in cultured cells, comprising the step of:

introducing an RNA thioaptamer into the cells in an amount sufficient to attenuate expression of the target gene, wherein the RNA thioaptamer comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene and mediates attenuation of protein expression for a gene to which it corresponds.

Claim 85. (Withdrawn) The method of claim 83, wherein the cell is in cell culture.

Claim 86. (Withdrawn) The method of claim 83, wherein the cell is infected with a virus.

Claim 87. (Withdrawn) The method of claim 83, wherein the cell is a mammalian cell.

Claim 88. (Withdrawn) The method of claim 83, wherein the cell is a human cell.

Claim 89. (Withdrawn) The method of claim 83, wherein the cell is a stem cell.

Claim 90. (Withdrawn) The method of claim 83, wherein the thioaptamer is an antisense molecule.

Claim 91. (Withdrawn) The method of claim 83, wherein the thioaptamer is a ribozyme.

Claim 92. (Withdrawn) The method of claim 83, wherein the thioaptamer is a double-stranded RNA (dsRNA).

Claim 93. (Withdrawn) The method of claim 83, wherein the gene is associated with a disease or disorder.

Claim 94. (Withdrawn) A method for attenuating expression of a target gene in a mammalian cell, comprising the steps of:

introducing into the cell a thioaptamer in an amount sufficient to attenuate expression of the target gene, wherein the thioaptamer mediates gene silencing of a nucleic acid to which it hybridizes under stringent conditions; and activating a gene silencing activity in the cell.

Claim 95. (Withdrawn) The method of claim 93, wherein the cell is in cell culture.

Claim 96. (Withdrawn) The method of claim 93, wherein the cell is in an animal.